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Ultrastructural Study of the Tetrathyridium of *Mesocestoides corti* Hoeppli, 1925 (Cestoda): The Regeneration of 2 - Sucker Head Fragments *

by

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With 15 figures

Abstract

Two-sucker head fragments of *M. corti* tetrathyridia regenerate the missing parts of their body. Primary wound closure takes place by muscle contraction, by the formation of a cicatrization syncytium from cell bodies of the tegumental syncytium, and by the strong adhesion among the lobes of glycogen-storing parenchyma cells. Definitive wound healing is however not achieved. The tail fragment bearing the wound is discarded. Degenerating cell fragments are phagocytized by calcareous corpuscule cells. Germinative cells and dedifferentiated glycogen-storing cells represent the main pool of cells needed for the progressive phase of regeneration. Parts of the tegument participate in the formation of the suckers.

INTRODUCTION

Hart (1968) described the regeneration of head fragments from tetrathyridia of *Mesocestoides corti in vivo* and *in vitro*. He found that fragments with at least 1 sucker successfully regenerated the lacking organs and developed into normal tetrathyridia capable of asexual multiplication. Experimentally produced fragments without suckers survived for a short time much in the same way as spontaneously detached tail fragments (Hess 1972), but were unable to regenerate. Hess & Guggenheim (1977) and Hess (1980, 1981) studied the fine structure of tetrathyridia of *M. corti* and the morphogensis of the tegument, the parenchyma, the internal muscles and the suckers during asexual mul-

* Part of the author's thesis.

tiplication. In this study, the reaction of tetrathyridia to injury and the reparative regeneration of 2-sucker head fragments is described.

MATERIALS AND METHODS

Tetrathyridia of *Mesocestoides corti* originally isolated by Specht & Voge (1965) and cultivated in our laboratory in NMRI-mice were used for this study. Tetrathyridia bearing four suckers were cut transversally behind the scolex in order to obtain 4-sucker head fragments which were cut sagitally to produce 2-sucker head fragments. The working solution used was warm NTCT 135 containing antibiotics (Voge & Coulombe 1966). Immediately after amputation, the 2-sucker head fragments were injected into the peritoneal cavity of NMRI-mice. Six hours, 15 hours, 3, 4, 5, 6, 7 and 10 days after injection, the regenerating fragments were collected from their hosts and fixed in 2% glutaraldehyde buffered by 0,1 M Na-cacodylate, pH 7, 2, at 4° C for 1 day and post-fixed in 1% OsO_4 buffered by 0,1 M Na-cacodylate, pH 7, 2, at room temperature for 90 minutes, dehydrated and embedded. The sections were studied on a Philips 201 EM.

RESULTS

The shock

The immediate reaction of the 2-sucker head fragments to amputation is a violent muscular contraction lasting for 5 to 15 minutes (Fig. 1). After relaxation, the fragments show normal mobility. The muscles of the wound region remain however contracted, thus holding the wound closed. The suckers of the fragments move convulsively and are projected as the longitudinal parenchymal muscles (sucker retractors) are cut.

The fragments 6 hours after amputation

Morphology : The 2-sucker head fragments are of oval or longish shape. The suckers are still evaginated. The wound surface is reduced to a small slit covered by a cap with hyaline appearance *in vivo* (Fig. 1).

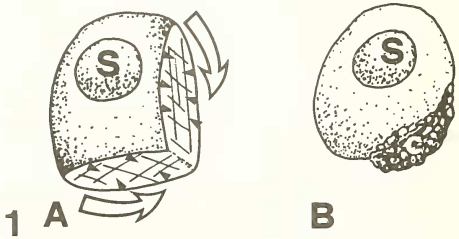

Fig. 1.

Tetrathyridium of *M. corti :* Schematic representation of the shock contraction of the 2-sucker head fragments. A: before; B: after contraction; Arrows: direction of contraction; S: sucker; c: cap of expulsed cells.

Fine structure

The tegumental syncytium: The tegumental syncytium opposite to the wound has a normal fine structure but the polynuclear cell mass of the apical massif which has been sectioned during amputation has disappeared. Towards the wound the superficial layer becomes thinner, the space between the microtriches is larger than normal, and the disc-shaped bodies are in a parallel position to the surface (normal position is perpendicular): the superficial cytoplasm seems to be stretched.

The tegument of the wound lips is relatively thick. This is due to the lasting contraction of the subtegumental muscles in the wound region. The superficial cytoplasm of this zone shows signs of degeneration. The zone of normal cytoplasm passes imperceptibly into a strongly vacuolated region which bears microvilli and finally into a layer of homogeneous granular appearance (Fig. 2). The homogeneous zone represents the lips of the wound. The degeneration of the tegumental superficial cytoplasm takes place in zones which have lost close contact with the perinuclear cell bodies. Some of the perinuclear cell bodies of the tegumental syncytium around the wound region fuse and form bi- and multinucleated cell masses (Fig. 3). In these cell bodies the large nuclei have a central nucleolus each, and the RER and the Golgi apparatus are well developed. The cytoplasm still contains vesicles filled with cristal-like membraneous aggregates which are typical for the perinuclear cell bodies of the normal tegumental syncytium (Hess, 1980).

In zones where the subtegumental muscle layer and the tegumental syncytium have been separated mechanically during amputation, the original structure of the latter is modified. In this case, the perinuclear cell bodies fuse with the superficial cytoplasm to form a unique nucleated layer (Fig. 4).

The subtegumental and parenchymal muscles: Cut muscle fibres of both the subtegumental and the parenchymal muscle systems decompose. In the perinuclear cell bodies of intact muscle cells an increased activity of the RER including formation of cisternae occurs. These cisternae release their content into the interstitial space (Fig. 5). The composition and function of the secretion is unknown. At the same time myoblasts are found in the parenchyma. They have long cytoplasmic processes in which myofibrils are synthesized as during asexual multiplication (Hess 1980).

Glycogen-storing parenchyma cells and calcareous corpuscule cells: A great number of glycogen-storing parenchyma cells and calcareous corpuscule cells have been expulsed during the shock contraction. After relaxation of the muscles, the remaining parenchymal glycogen-storing cells which are joined together by gap junctions form a loose network. The striking feature of the parenchyma of 6 hour old fragments was the presence of large interstitial spaces probably due to increased osmolality (Figs. 3-8). Glycogen-storing lobes of parenchyma cells encapsulate or envelop decomposing cell fragments (i.e. muscle fibres) but they do not phagocytize them. The phagocytosis of decomposing fragments takes place by calcareous corpuscule cells. These cells have long cytoplasmic filaments or lamellae (ruffle membranes ?) which pick decomposing fragments out from the envelopes formed by the glycogen-storing parenchyma cells and phagocytize them (Figs. 6, 7, 8). The phagosomes advance in the cytoplasmic filaments towards the calcareous corpuscule and are incorporated in the latter. During the migration of the phgaosome, homogeneization of the content occurs. In the cytoplasm of phagocytizing calcareous corpuscule cells one finds vesicles with electron-dense granules, possibly primary lysosomes (Fig. 7). These vesicles seem to fuse with the phagosomes which migrate towards the calcareous corpuscule (Fig. 8).

Some of the parenchymal glycogen-storing cells undergo dedifferentiation. In such cells plasmalemma formation takes place between the ribosome-containing perinuclear cytoplasm and the glycogen-storing lobes (Fig. 9). Subsequently, the glycogen-storing lobes detach from the nucleated cell body. Numerous free lobes accumulate in the interstitial space adhering together by gap-junctions (Figs. 10, 14). The dedifferentiated cells are similar to germinative cells. They have a large nucleus and their cytoplasm contains only free ribosomes and some small mitochondria (Fig. 10).

Germinative cells: The mitotic activity of the germinative cells does not seem to be increased; no quantitative analysis has however been undertaken. Many of the germinative cells differenciate into muscle cells. Dark cells which form components of the osmoregulatory or excretory system (protonephridia, canal cells) probably also derive from germinative cells.

The cap: The wound surface is covered by a cap composed of different types of cells and cell fragments, mainly glycogen-containing lobes and muslces fibres (Fig. 11). Some of the cells remain alive but seem to dedifferentiate, most of them vacuolize and decompose as do the cell fragments.

The fragments 15 hours after amputation

The morphology of 15 hour old fragments is the same as 6 hour ones.

Fine structure

Tegumental syncytium: In the perinuclear cell bodies of the whole larva an accumulation of lipid droplets is observed. In the zones opposite the wound, no other modifications are observed. In the wound region, the formation of a syncytial cell mass from tegumental perinuclear cell bodies continues. We call it the cicatrization syncytium (Fig. 12). It remains in cytoplasmic continuity with the tegumental syncytium. Its cytoplasm contains a great number of free ribosomes, mitochondria and well developed Golgi apparatus and SER. The RER seems to decrease and the electron-dense vesicles with membrane aggregates which are normally found in the tegumental syncytium have disappeared. Each nucleus contains a large central nucleolus and the heterochromatine is finely scattered throughout the nucleoplasm. Mitotic divisions are not observed. The cicatrization syncytium forms a network which separates the parenchyma from the cap. This wound closure is however incomplete and the cicatrization syncytium never transforms into normal tegument.

Parenchyma: In the wound region, the glycogen-storing parenchyma cells divide into a large number of fragments. The gap junctions which held these small glycogen-storing lobes together are so numerous that they are arranged side by side (Fig. 14). It is possible that the fragmentation of the glycogen-storing lobes is related to the dedifferentiation of the glycogen-storing parenchyma cells.

The fragments 3 to 10 days after amputation

Three days after amputation most of the 2-sucker head fragments have begun the progressive phase of regeneration (definition see page 939). Large individual differences concerning the speed of regeneration are observed. Some fragments develop sucker anlagen after 3 days, while others show first signs of differentiation only after 10 days.

In the anterior part of the fragments a blastema is formed by undifferentiated cells. It is impossible to decide whether the blastema cells derive from the apical massif or from the pool of germinative cells.

Tegument: The apical massif which had disappeared during the first days after amputation reappears when the sucker anlagen are formed. It is situated between the original and the regenerating suckers. Its firm structure is the same as in non-dividing tetrathyridia (Hess 1980). Some days after amputation, the 2-sucker head fragments begin to grow. The tegumental growth takes place by the integration of migrating cells into the tegumental syncytium behind the suckers. This mode of tegumental growth has also been observed in normally developing tetrathyridia (Hess 1980).

The cicatrization syncytium which is formed in the wound region by the tegumental syncytium closes the wound incompletely. It never transforms into normal tegument with microtriches, thus it is unable to restore the body surface of the animal. The reconstitution of the tegument takes place indirectly. When the fragments have achieved a certain length or degree of development, the posterior part of the animal is eliminated by transversal fission. This is a normal way used by the tetrathyridia to reduce their body length and to eliminate the aged posterior part of their body (Hess 1972, 1980). After transversal fission, the superficial cytoplasm of the tegumental syncytium invaginates and fuses with the main excretory canals thus forming the terminal vesicle(s). In this way, normal reconstitution of the body surface is achieved.

The suckers: The sucker anlagen are visible by light microscopy at the earliest 3 days after the operation. In the electron microscope different cell types composing the sucker blastema are distinguishable.

1. The first differentiated cells which appear are fibroblasts with very long pseudopods. They form a cup the rim of which touches the fibrous layer of the tegument (Fig. 13). This cup separates the sucker anlage from the surrounding scolex blastema. The fibrous layer of the sucker is secreted when the differentiation of the myoblasts is advanced.

2. Myoblasts with long cytoplasmic expansions differentiate in the blastema (Fig. 15). They develop RER-cisternae and form the muscle fibres of the perpendicular, transversal lower and longitudinal lower muscle systems. The transversal and the longitudinal upper sucker muscles represent transformed circular and longitudinal subtegumental muscles (Fig. 13) (see Hess, 1981).

3. Cells which accumulate alpha-glycogen are scattered throughout the anlagen. They grow and develop into glycogen-storing sucker cells (Fig. 15).

4. The cell bodies of the tegumental syncytium are slightly electron-denser than the cells named above and their cytoplasm contains well developed RER and Golgi apparatus (Fig. 15). During the sucker morphogenesis, the cytoplasmic bridges between the superficial cytoplasm and the perinuclear cell bodies disappear and the latter fuse to form the interstitial syncytium.

5. Neurons seem to differentiate late. Their development has not been studied in detail.

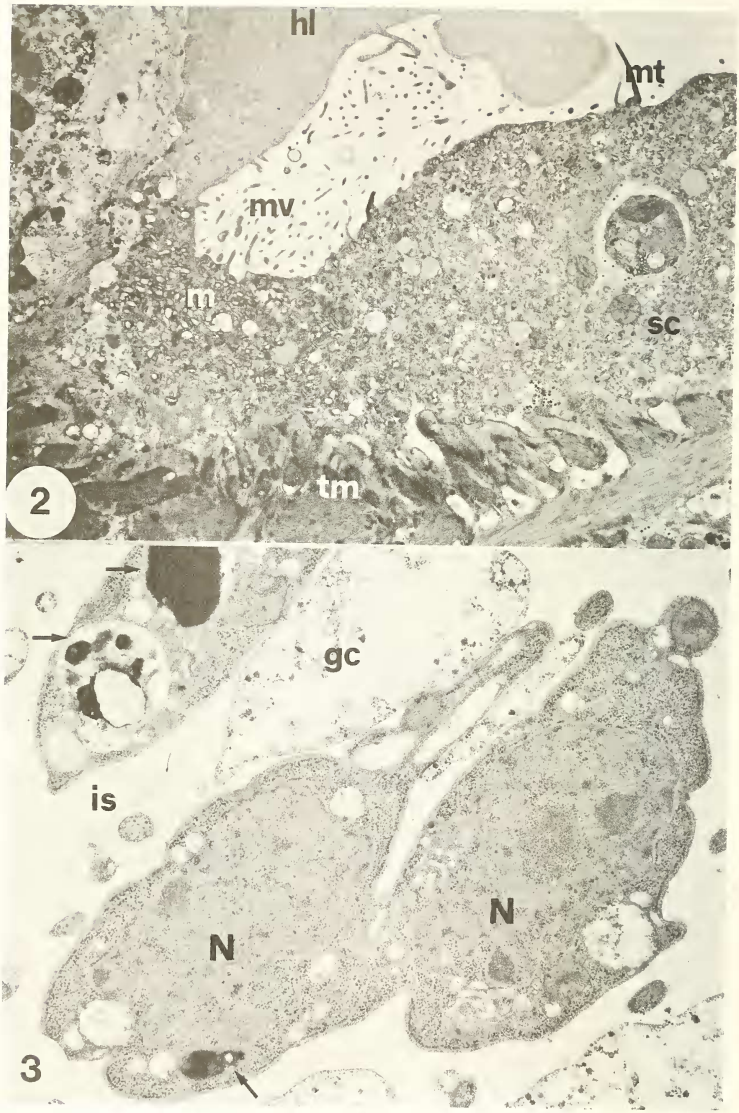
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FIG. 2.

Tetrathyridium of *M. corti*: Section of the tegumental wound lip. Between the superficial cytoplasm of relatively normal appearance (sc) and the degenerated homogeneous layer (hl) extends a zone of vacuolated microvilli-bearing cytoplasm (m). mv:microvilli; tm: subtegumental muscles. 5770×

FIG. 3.

Tetrathyridium of *M. corti:* Binucleated cell bodies of the tegumental syncytium near the wound lip. N: nuclei; arrows: vesicles containing cristal-like inclusions; gc: glycogen-storing parenchyma cell; is: interstitial space. 16,870×

FIG. 4.

Tetrathyridium of *M. corti:* Transverse section of the tegumental syncytium near the wound lip having lost the original organization. The perinuclear cell bodies (N: nuclei) have fused with the superficial cytoplasm (sc). is: interstitial space. 5780×.

FIG. 5.

Tetrathyridium of *M. corti*: Cell body of a myocyte with well developed RER cisternae (c); is: interstitial space. 26,200×. Inset: Secretion of the content of a RER cisterna (c) into the interstitial space. 56,680×.

FIG. 6.

Tetrathyridium of *M. corti*: Cytoplasmic filaments or lamellae (arrows) of a calcareous corpuscule cell isolate and phagocytize a degenerating muscle fragment (df) from its envelope formed by a glycogenstoring parenchyma cell (gc). 18,670×.

FIG. 7.

Tetrathyridium of *M. corti*: Phagosome (ps) enclosed in a cytoplasmic expansion of a calcareous corpuscule cell; cc: calcareous corpuscule; arrows: primary lysosome-like vesicle. 26,870×.

FIG. 8.

Tetrathyridium of *M. corti*: Vacuole (ps) containing electron-dense granules (arrow) (autophagic vesicle ?) situated near the calcareous corpuscule (cc). 26,870×.


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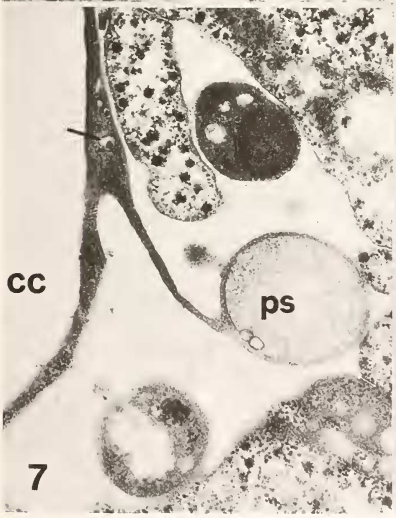
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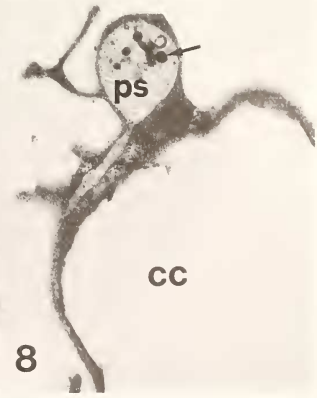
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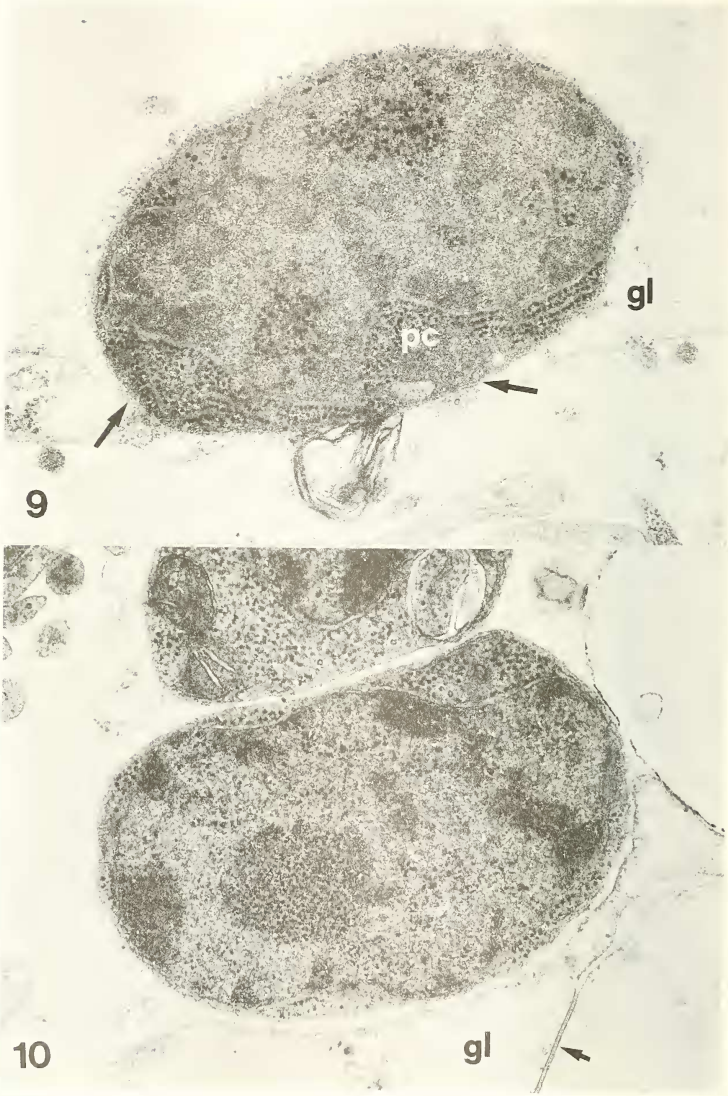
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FIG. 9.

Tetrathyridium of *M. corti*: Dedifferentiating parenchymal glycogen-storing cell. Plasmalemma formation (arrows) takes place between the perinuclear cytoplasm (pc) and the glycogen-storing lobes (gl). 43,490×.

FIG. 10.

Tetrathyridium of *M. corti*: The dedifferentiated glycogen-storing cell has a fine structure similar to a germinative cell; gl: glycogen-storing lobe; arrow: gap-junction. 43,490×.

FIG. 11.

Tetrathyridium of *M. corti*:
Section of the cap with expulsed cells and cell fragments. 5660×.

FIG. 12.

Tetrathyridium of *M. corti*: Part of the cicatrization syncytium. N: nuclei; l: lipid droplets; arrows: zones of Golgi-apparatus and/or SER. 8180×.

FIG. 13.

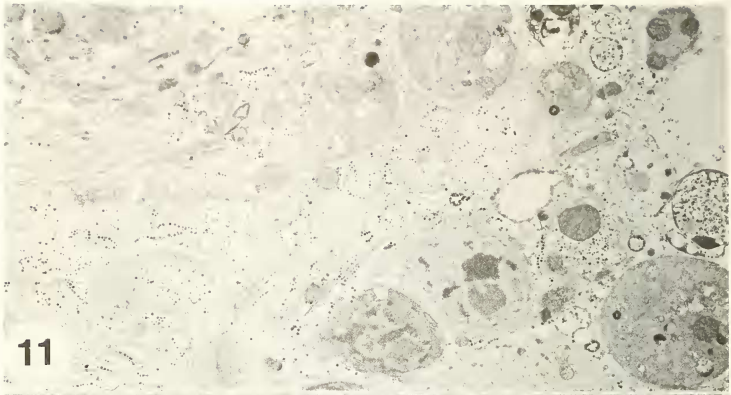
Tetrathyridium of *M. corti*: Sucker anlage of a 3 day old fragment. Fibroblasts (fb, arrows) separate the sucker anlagen (S) from the parenchymal part (P) of the scolex blastema. The sucker anlage contains undifferentiated cells (uc) and precursors of the interstitial syncytium (is) which are transformed perinuclear cell bodies of the tegumental syncytium detaching from the superficial cytoplasm (sc); asterisk: subtegumental muscles transforming into the transversal upper muscle system of the sucker. 4225×.

FIG. 14.

Tetrathyridium of *M. corti*: Glycogen-storing lobes of the wound region adhering together by numerous gap-junctions (arrows). 38,020×.

FIG. 15.

Tetrathyridium of *M. corti*: Section of a differentiating sucker anlage with undifferentiated cells (uc), myoblasts (mb), and glycogen-storing cells (asterisk). The cell bodies of the developing interstitial syncytium (is) may still have cytoplasmic continuity (arrows) with the superficial cytoplasm of the tegumental syncytium (sc). u: upper transversal and longitudinal sucker muscles deriving from the subtegumental muscles. 5535×.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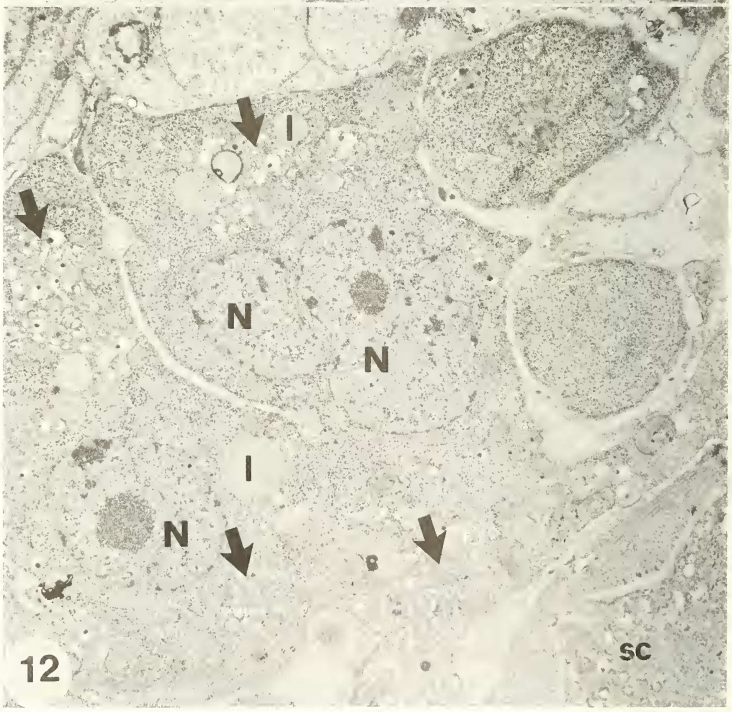
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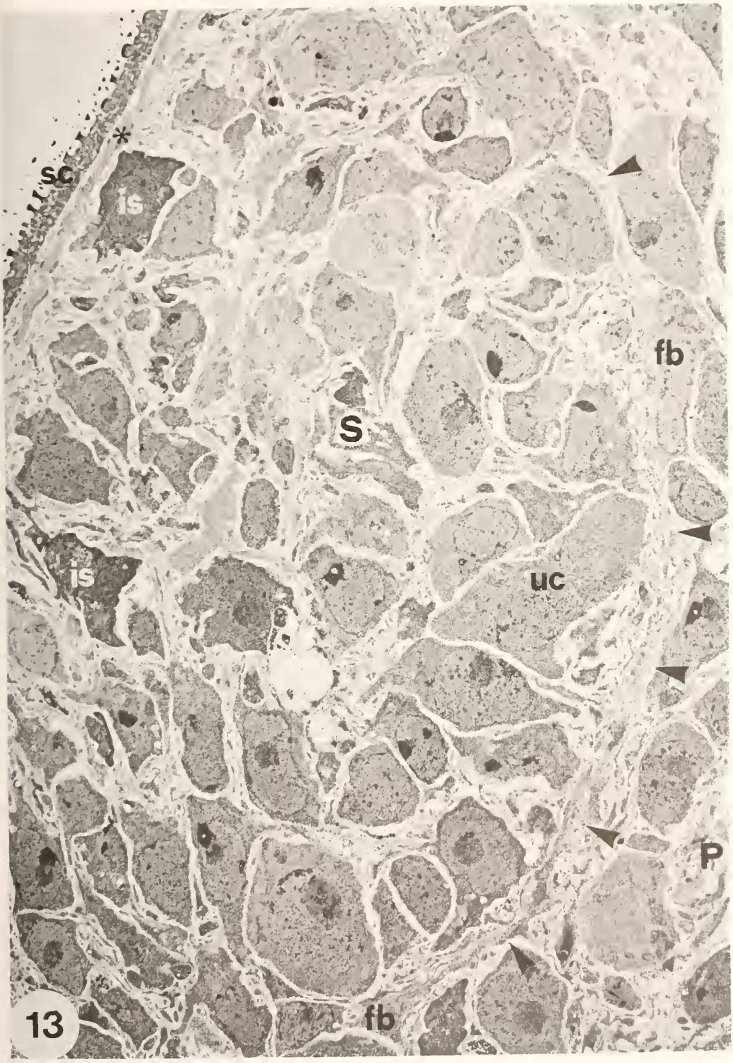
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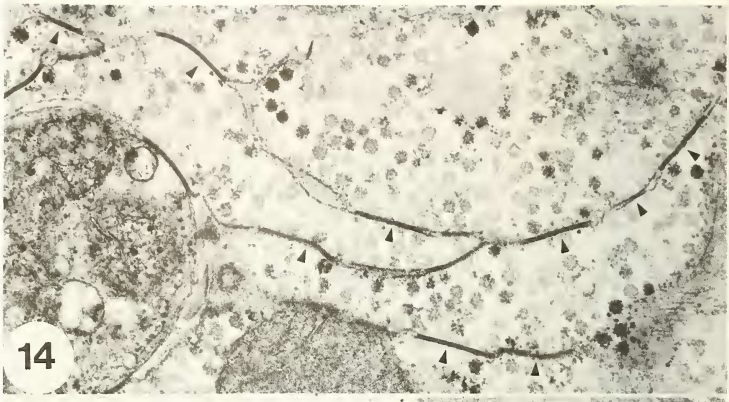
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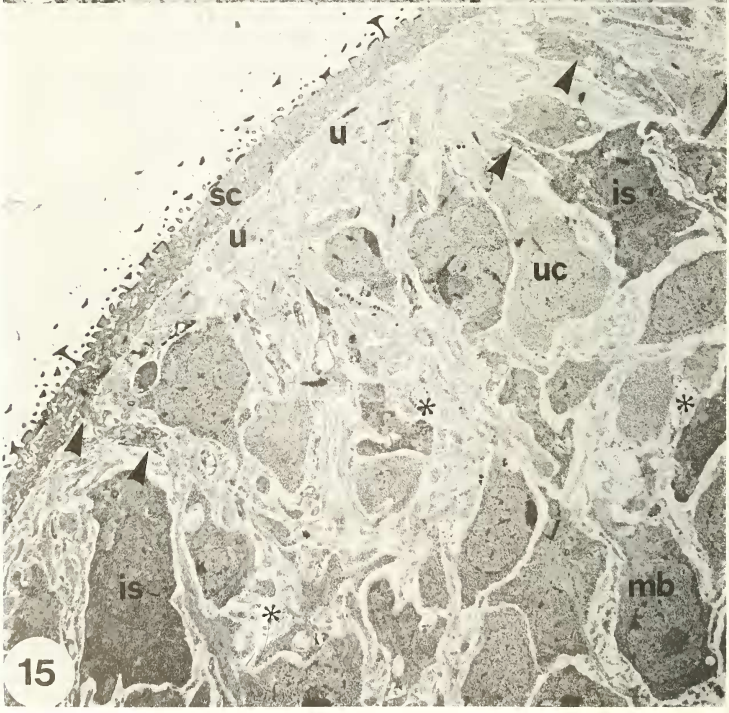
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DISCUSSION AND CONCLUSIONS

According to NEEDHAM (1952) every process of reparative regeneration is divided into a regressive phase and a progressive phase. The regressive phase is induced by the trauma and comprises i) wound closure, ii) destruction of injured cells, iii) defense against infection and toxic substances, iv) predetermination of the cells needed for the progressive phase. The progressive phase comprises i) the formation, ii) the growth, and iii) the differentiation of the blastema. In this study, NEEDHAM's theory is confirmed about regenerating 2 sucker head fragments of tetrathyridia of *M. corti.*

The initial wound closure takes place by the shock contraction of all muscle systems which draw the tegumental wound edges together. The shock contraction also expulses a great number of parenchymal cells. thus reducing the volume of the parenchyma. Subsequently, the muscles relax except the subtegumental muscles of the wound region. Thus the body surface increases and the superficial cytoplasm of the tegumental syncytium is stretched, which is conspicuous by the position of the disc shaped bodies parallel to the larval surface. The tegumental extension seems to be a passive one, possibly due to an increase of the osmotic pressure of the interstitial fluid. This would also explain the loose structure of the parenchyma.

A second mechanism of wound closure is the formation of the cicatrization syncytium between the cap of expulsed cells and the parenchyma. It has to be pointed out that the cicatrization syncytium is a part of the tegumental syncytium. The failure of the cicatrization syncytium to transform into normal tegument with microtriches underlines the antero-posterior determination of the tegumental syncytium (HESS 1980). The definite healing of the wound is achieved indirectly by discarding the posterior part of the body including the wound. The elimination of a tail fragment occurs periodically in *M. corti* tetrathyridia which have to be considered as continuously growing metacestodes.

The glycogen-storing parenchyma cells have a triple function during reparative regeneration. By dedifferentiation, they produce cells which are cytologically identical to germinative cells and which are supposed to have high histogenetic potency. Thus they contribute also to the progressive phase of regeneration. In the wound region, the glycogen-storing lobes of parenchyma cells divide into numerous small fragments adhering together by a great number of gap junctions. This reaction which is probably related to dedifferentiation could work against stress in the wound region and protect the parenchyma against external influences. Glycogen-storing parenchyma cells also envelop degenerating cell fragments thus isolating them from the uninjured tissue.

The final elimination of cell fragments occurs by calcareous corpuscule cells. The capacity of phagocytosis of these cells has never been described before. This observation may be important as the role of the calcareous corpuscule cells is still a matter of conjecture (for review see SMYTH 1969). The experimental model used here could contribute towards the examination of the functions of the calcareous corpuscule cells or at least to studying exhaustively one of their capacities. At any rate our observations seem to support the hypothesis that calcareous corpuscules have to be considered as a kind of residual body.

The anterior inviginated part of the tegumental syncytium, called the apical massif (HESS 1980), is histogenetically the most active part of the tegumental syncytium during asexual multiplication. It differentiates into tegumental syncytium, subtegumental muscles, glycogen-storing parenchyma cells and parts of the suckers. During reparative regeneration the apical massif disappears histologically. It is not clear if it transforms entirely into tegument or if it contributes also to the formation of the scolex blastema

from which the suckers derive. It is however evident that parts of the suckers, i.e. the interstitial syncytium and the upper sucker muscles derive directly from the tegumental syncytium and the subtegumental muscles, thus they are indirect products of the apical massif. This observation confirms the description of the morphogenesis of the suckers during asexual multiplication (Hess 1981). After the formation of the sucker anlagen, the apical massif reappears. Histologically it is probable that, from this moment on, it forms new superficial cytoplasm and differentiates into subtegumental muscles and parenchymal glycogen-storing cells as during asexual multiplication (Hess 1980).

In conclusion, the ability to reparative regeneration is well developed in *M. corti* tetrathyridia and closely related to its ability of asexual multiplication as seen by the reaction of the tissues.

The fact that only scolex-bearing fragments regenerate a complete tetrathyridium is probably a consequence of the anatomy of these animals. The main nervous ganglia as well as the apical massif is situated near the suckers (Hart 1968). Thus each single sucker head fragment contains a part of the central nervous system which is known to play the most important role for all processes of typical regeneration.

Acknowledgements

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